DNA as Biological Information

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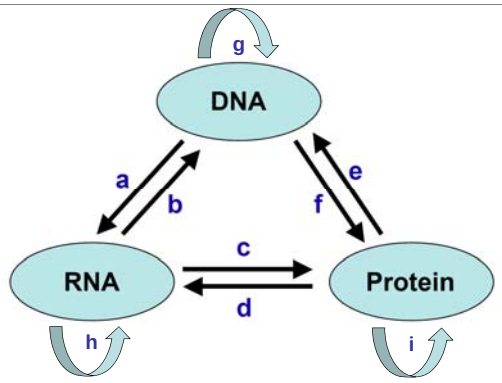
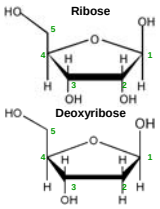
Overview

- Learning objectives
  - Biological information
  - DNA sequencing techniques and DNA data
  - File formats used for biological data
  - Introduction to the GenBank database

Information flow in biological systems

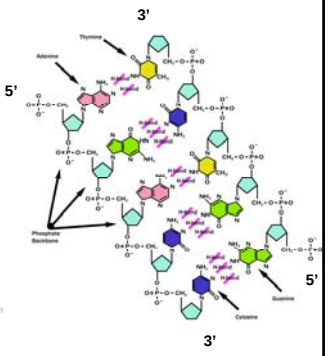


DNA sequences = summary of information



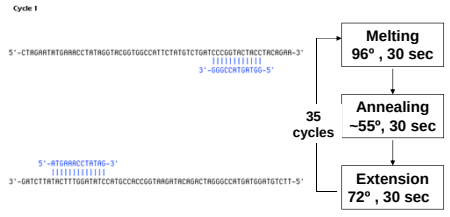
5’ AGCC 3’
3’ TCGG 5’

5’ ATGGCCAGGTAA 3’



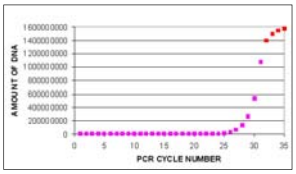
DNA backbone: http://en.wikipedia.org/wiki/DNA
(Deoxy)ribose: http://en.wikipedia.org/

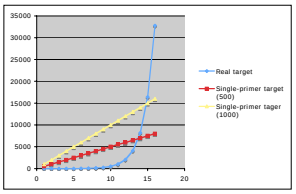
PCR



Animation: http://depts.washington.edu/~genetics/courses/genet371b-aut99/PCR_contents.html

PCR

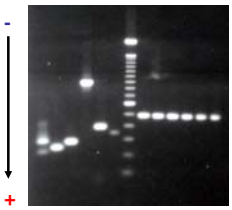




Animation: http://www.people.virginia.edu/~rjh9u/pcranim.html
PCR graph: http://pathmicro.med.sc.edu/pcr/realtime-home.htm

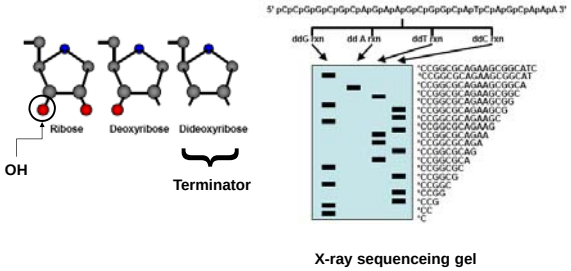
Gel electrophoresis

- DNA fragments are separated using gel electrophoresis
  - Typically 1% agarose
  - Colored with EtBr or ZybrGreen (glows in UV light).
  - A DNA "ladder" is used for identification of known DNA lengths.



Gel picture: http://www.pharmaceutical-technology.com/projects/roche/images/roche3.jpg
PCR setup: http://arbl.cvmbs.colostate.edu/hbooks/genetics/biotech/gels/agardna.html

The Sanger method of DNA sequencing

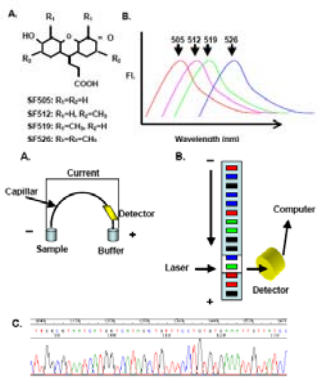


X-ray sequenceing gel

Images: http://www.idtdna.com/support/technical/TechnicalBulletinPDF/DNA_Sequencing.pdf

Automated sequencing

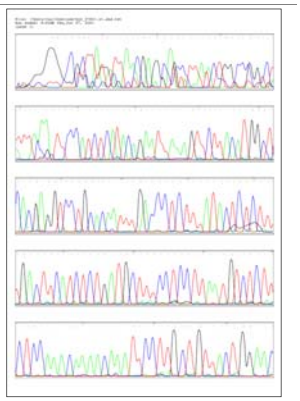
- The major break-through of sequencing has happened through *automation.*
- Fluorescent dyes.
- Laser based scanning.
- Capillary electrophoresis
- Computer based base-calling and assembly.



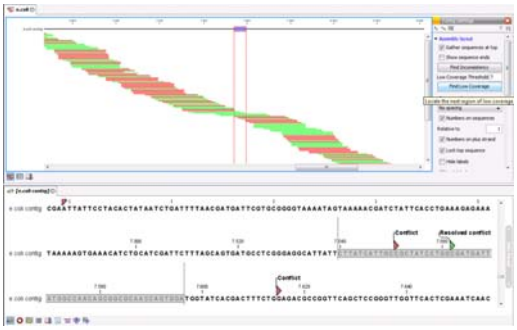
Images: http://www.idtdna.com/support/technical/TechnicalBulletinPDF/DNA_Sequencing.pdf

Handout exercise: ”base-calling”

- Handout: Chromatogram
- Groups of 2-3.
- Tasks:
  - Identify “difficult” regions
  - Identify likely errors
  - Try to estimate the best interval to use



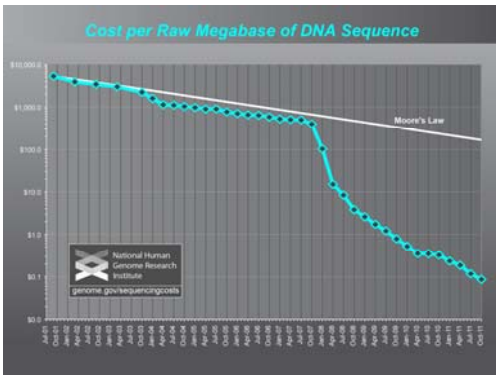
Sequence read mapping



DNA sequencing – history

1972 Recombinant DNA technology [Paul Berg].
1976 The first sequenced genome, the bacteriophage MS2 [Walter Fiers *et al*.]
1977 DNA sequencing by chemical cleavage [Allan Maxam & Walter Gilbert]; DNA sequencing by enzymatic synthesis [Fred Sanger].
1982 *GenBank* (public data base of DNA sequences).
1987 The first automatic sequencing machine, *Prism 373* [Applied Biosystems].
1990 The *Human Genome Project* is launched.
1995 The first genome of a free-living organism, the bacterium *Haemophilus influenzae* (1.8 Mb) [The Institute for Genomic Research (TIGR)].
1996 The first genome of a eukaryote, Baker’s yeast, *Saccharomyces cerevisiae* (12.1 Mb) [International consortium].
1998 The first genome of an animal, the worm *Caenorhabditis elegans* (97Mb) [Sanger Center and partners].
2001 The first “drafts” of the human genome (3Gb) [Human Genome Project Consortium (Nature, 15 Feb) + Celera (Science, 16 Feb)].
April 11, 2013 *GenBank release 195* contains 164,136,731 sequences with a total of 151,178,979,155 nucleotides (the files take up 594 GB).

Cost of sequencing



Background - Nucleotide databases

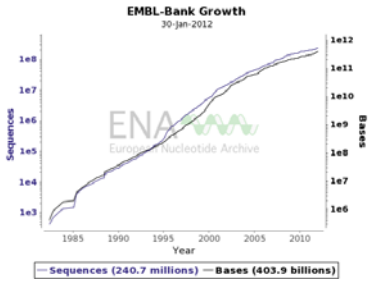
- **GenBank**, http://www.ncbi.nlm.nih.gov/Genbank/
- National Center for Biotechnology Information (NCBI), National Library of Medicine (NLM), National Institutes of Health (NIH), USA
- Established in 1982.
- **EMBL**, http://www.ebi.ac.uk/embl/
- European Bioinformatics Institute (EBI), England
- Established in 1980 by the European Molecular Biology Laboratory, Heidelberg, Germany
- Now part of **ENA**, the European Nucleotide Archive, http://www.ebi.ac.uk/ena/
- **DDBJ**, http://www.ddbj.nig.ac.jp/
- National Institute of Genetics, Japan

- *Together they form*
- International Nucleotide Sequence Database Collaboration, http://www.insdc.org/

INSDC International Nucleotide Sequence Database Collaboration

Nucleotide database growth

- Growth is roughly exponential
- But doubling time has increased from ~20 months (1990s) to ~50 months (2010)
- NB: The databases are public — no restrictions on the use of the data within.



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FASTA format

```
>alpha-D
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CCAGACTGTGGAGCCGAGGCCCTGGAGAGGTGCGGGCTGAGCTTGGGGAAACCATGGGCA
AGGGGGGCGACTGGGTGGGAGCCCTACAGGGCTGCTGGGGGTTGTTCGGCTGGGGGTCAG
CACTGACCATCCCGCTCCCGCAGCTGTTCACCACCTACCCCCAGACCAAGACCTACTTCC
CCCACTTCGACTTGCACCATGGCTCCGACCAGGTCCGCAACCACGGCAAGAAGGTGTTGG
CCGCCTTGGGCAACGCTGTCAAGAGCCTGGGCAACCTCAGCCAAGCCCTGTCTGACCTCA
GCGACCTGCATGCCTACAACCTGCGTGTCGACCCTGTCAACTTCAAGGCAGGCGGGGGAC
GGGGGTCAGGGGCCGGGGAGTTGGGGGCCAGGGACCTGGTTGGGGATCCGGGGCCATGCC
GGCGGTACTGAGCCCTGTTTTGCCTTGCAGCTGCTGGCGCAGTGCTTCCACGTGGTGCTG
GCCACACACCTGGGCAACGACTACACCCGGAGGCATGCTGCCTTCGACAAGTTCCTG
TCGGCTGTGTGCACCGTGCTGGCCGAGAAGTACAGATAA
>alpha-A
ATGGTGCTGTCTGCCAACGACAAGAGCAACGTGAAGGCCGTCTTCGGCAAAATCGGCGGC
CAGGCCGGTGACTTGGGTGGTGAAGCCCTGGAGAGGTATGTGGTCATCCGTCATTACCCC
ATCTCTTGTCTGTCTGTGACTCCATCCCATCTGCCCCCATACTCTCCCCATCCATAACTG
TCCCTGTTCTATGTGGCCCTGGCTCTGTCTCATCTGTCCCCAACTGTCCCTGATTGCCTC
TGTCCCCCAGGTTGTTCATCACCTACCCCCAGACCAAGACCTACTTCCCCCACTTCGACC
TGTCACATGGCTCCGCTCAGATCAAGGGGCACGGCAAGAAGGTGGCGGAGGCACTGGTTG
AGGCTGCCAACCACATCGATGACATCGCTGGTGCCCTCTCCAAGCTGAGCGACCTCCACG
CCCAAAAGCTCCGTGTGGACCCCGTCAACTTCAAAGTGAGCATCTGGGAAGGGGTGACCA
GTCTGGCTCCCCCTCCTGCACACACCTCTGGCTACCCCCTCACCTCACCCCCTTGCTCACC
ATCTCCTTTTGCCTTTCAGCTGCTGGGTCACTGCTTCCTGGTGGTCGTGGCCGTCCACTT
CCCCTCTCTCCTGACCCCGGAGGTCCATGCTTCCCTGGACAAGTTCGTGTGTGCCGTGGG
CACCGTCCTTACTGCCAAGTACCGTTAA
```

(Handout)

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GenBank format

- Originates from the GenBank database.
- Contains both a DNA sequence and annotations of features (*e.g.* location of genes).

(handout)

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GenBank format - HEADER

```
LOCUS       CMGLOAD                1185 bp    DNA     linear   VRT 18-APR-2005
DEFINITION  Cairina moschata (duck) gene for alpha-D globin.
ACCESSION   X01831
VERSION     X01831.1  GI:62724
KEYWORDS    alpha-globin; globin.
SOURCE      Cairina moschata (Muscovy duck)
  ORGANISM  Cairina moschata
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Archosauria; Aves; Neognathae; Anseriformes; Anatidae; Cairina.
REFERENCE   1  (bases 1 to 1185)
  AUTHORS   Erbil,C. and Niessing,J.
  TITLE     The primary structure of the duck alpha D-globin gene: an unusual
            5' splice junction sequence
  JOURNAL   EMBO J. 2 (8), 1339-1343 (1983)
   PUBMED   10872328
COMMENT     Data kindly reviewed (13-NOV-1985) by J. Niessing.
```

GenBank format - ORIGIN section

```
ORIGIN
        1 ctgcgtggcc tcagcccctc cacccctcca cgctgataag ataaggccag ggcgggagcg
       61 cagggtgcta taagagctcg gccccgcggg tgtctccacc acagaaaccc gtcagttgcc
      121 agcctgccac gccgctgccg ccatgctgac cgccgaggac aagaagctca tcgtgcaggt
      181 gtgggagaag gtggctggcc accaggagga attcggaagt gaagctctgc agaggtgtgg
      241 gctgggccca gggggcactc acagggtggg cagcagggag caggagccct gcagcgggtg
      301 tgggctggga cccagagcgc cacgggggtgc gggctgagat gggcaaagca gcagggcacc
      361 aaaactgact ggcctcgctc cggcaggatg ttcctcgcct acccccagac caagacctac
      421 ttccccccact tcgacctgca tcccggctct gaacaggtcc gtggccatgg caagaaagtg
      481 gcggctgccc tgggcaatgc cgtgaagagc ctggacaacc tcagccaggc cctgtctgag
      541 ctcagcaacc tgcatgccta caacctgcgt gttgaccctg tcaacttcaa ggcaagcggg
      601 gactagggtc cttgggtctg ggggtctgag ggtgtggggt gcagggtctg ggggtccagg
      661 ggtctgagtt tcctgggggtc tggcagtcct gggggctgag ggccagggtc ctgtggtctt
      721 gggtaccagg gtcctgggggg ccagcagcca gacagcaggg gctgggattg catctgggat
      781 gtgggccaga ggctgggatt gtgtttggaa tgggagctgg gcaggggcta gggccagggt
      841 gggggactca gggcctcagg gggactcggg gggggactga gggagactca gggccatctg
      901 tccggagcag gggtactaag ccctggtttg ccttgcagct gctggcacag tgcttccagg
      961 tggtgctggc cgcacacctg ggcaaagact acagccccga gatgcatgct gcctttgaca
     1021 agttcttgtc cgccgtggct gccgtgctgg ctgaaaagta cagatgagcc actgcctgca
     1081 cccttgcacc ttcaataaag acaccattac cacagctctg tgtctgtgtg tgctgggact
     1141 gggcatcggg ggtcccaggg agggctgggt tgcttccaca catcc
//
```

GenBank format - FEATURE section

```
FEATURES             Location/Qualifiers
     source          1..1185
                     /organism="Cairina moschata"
                     /mol_type="genomic DNA"
                     /db_xref="taxon:8855"
     CAAT_signal     20..24
     TATA_signal     69..73
     precursor_RNA   101..1114
                     /note="primary transcript"
     exon            101..234
                     /number=1
     CDS             join(143..234,387..591,939..1067)
                     /codon_start=1
                     /product="alpha D-globin"
                     /protein_id="CAA25966.2"
                     /db_xref="GI:4455876"
                     /db_xref="GOA:P02003"
                     /db_xref="InterPro:IPR000971"
                     /db_xref="InterPro:IPR002338"
                     /db_xref="InterPro:IPR002340"
                     /db_xref="InterPro:IPR009050"
                     /db_xref="UniProt/Swiss-Prot:P02003"
                     /translation="MLTAEDKKLIVQVWEKVAGHQEEFGSEALQRMFLAYPQTKTYFP
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                     QCFQVVLAAHLGKDYSPEMHAAFDKFLSAVAAVLAEKYR"
     repeat_region   227..246
                     /note="direct repeat 1"
     intron          235..386
                     /number=1
     repeat_region   289..309
                     /note="direct repeat 1"
     exon            387..591
                     /number=2
     intron          592..939
                     /number=2
     exon            940..1114
                     /number=3
     polyA_signal    1095..1100
     polyA_signal    1114
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Exercise: GenBank

- The exercise guide is linked from the course programme.
- Read the guide carefully - it contains a lot of information about GenBank.
- Remember your handouts:
  - GenBank & FASTA format
  - Eukaryotic gene structure